Bronchoalveolar Cell Profiles in Children with Asthma, Infantile Wheeze, Chronic Cough, or Cystic Fibrosis

C. MARGUET, F. JOUEN-BOEDES, T. P. DEAN, and J. O. WARNER

Paediatric Respiratory Disease Unit, Hopital Ch. Nicolle, Rouen, France; and University Child Health, Southampton, United Kingdom

Differential cell counts of bronchoalveolar lavage (BAL) have been reported in normal children but few data on cellular profiles in bronchial diseases in childhood are available. We determined the BAL cell profiles of 72 children divided into 5 groups: asthma (n = 14), chronic cough (n = 12), infantile wheeze (n = 26), cystic fibrosis (n = 10), and control (n = 10). The highest total cell, eosinophil, and neutrophil counts were found in children with cystic fibrosis. The cell profile of children with chronic cough was similar to that of control children. Asthma and infantile wheeze were characterized by a high median ratio of eosinophils (3%) and neutrophils (12%), respectively. In both diseases, epithelial shedding was suggested by an elevated epithelial cell count, 13.5 and 12%, respectively. Lymphocyte subset analysis showed a higher proportion of CD8 cells (58 versus 40%) and therefore a lower CD4/CD8 ratio (0.266 versus 0.455) in children with asthma compared with infantile wheezers ($p = 0.02$). Irrespective of the presence or absence of radiological abnormalities, a proportion of neutrophils > 10%, was found in one-third of the children with asthma and in half of the infantile wheezers, and was related to symptom severity. We suggest that neutrophil-mediated inflammation, with or without bacterial infection, may contribute to symptoms of asthma in childhood. Chronic cough, however, is not associated with the cell profiles suggestive of asthma and in isolation should not be treated with prophylactic antiasthma drugs. Marguet C, Jouen-Boedes F, Dean TP, Warner JO. Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough, or cystic fibrosis.

AM J RESPIR CRIT CARE MED 1999;159:1533–1540.

Flexible fiberoptic bronchoscopy has become an important tool in the investigation of infants and children with airway diseases (1), and the main clinical indications are now well defined (2). Bronchoalveolar lavage (BAL) is a routine part of the procedure, primarily for microbiology but also to study cellular and noncellular factors associated with inflammation. In adults, BAL studies have improved the understanding of the pathophysiology of bronchial diseases and particularly asthma (3). BAL cell profiles have been defined in children with nonpulmonary illness (4–6) and in infants who underwent fiberoptic bronchoscopy for stridor or follow-up observation after the removal of a foreign body (7). However, relatively few data are available on the characteristics of BAL from children with asthma (8–12) or infantile wheeze (8, 9). To elaborate further on the pathophysiology of wheeze in childhood, we have conducted a study to determine the cellular profiles of BAL from children with a range of respiratory symptoms and signs, who needed bronchoscopy for clinical reasons. The cytologic findings have been assessed in relation to the microbiological, clinical, and radiological data.

(Received in original form May 11, 1998 and in revised form December 29, 1998)

Correspondence and requests for reprints should be addressed to Professor J. O. Warner, Child Health, Mailpoint 803, Southampton General Hospital, Southampton SO16 6YD, Hampshire, UK.

Am J Respir Crit Care Med Vol 159. pp 1533–1540, 1999
Internet address: www.atsjournals.org

METHODS

Subjects

Fiberoptic bronchoscopy was performed on 72 children divided into 5 groups with regard to age and diagnosis. Members of the first group (n = 14, 4–15 yr) were asthmatic (A), with a history of recurrent wheezing and dyspnea attacks with proven β_2-agonist reversible air flow limitation. The children in group CC (n = 12, 10 mo–13 yr) had isolated chronic cough, which has been suggested as a potential symptom of asthma (13). The third group (n = 26, 5–46 mo) consisted of infantile wheezers (W). All had at least three successive episodes of wheeze and cough during viral infections. The fourth group (CF) consisted of 10 children with cystic fibrosis (2.5–15 yr). All but one had a Shwachman score (SC) between 85 and 95, and FEV_1 higher than 75% of the expected value. One of the two children colonized with a *Pseudomonas* sp. had severe disease (SC, 68; FEV_1, 49%). Members of the final group (C) acted as controls (n = 10, 1.5–15 yr). These children had no identifiable lower airway disease. Written informed consent was obtained from the parents and the additional use of lavage specimens for research purposes was approved by the hospitals' ethics committees.

Clinical history of the chest disease, family history, allergy data, and chest radiographic features were recorded for each patient. Skin prick tests were routinely performed on children in groups A, CC, and W. The bronchoscopy indications are reported in Table 1. All of the children with CF had unsatisfactory clinical outcomes, and underwent the endoscopy in order to have a clear-cut microbiologic diagnosis. Twenty-nine children, including 3 with CF, had received inhaled steroids for at least 1 mo preceding the investigation and 2 with CF were receiving oral steroid (prednisolone, 1 mg/kg/d). We classified the patients of the groups A, CC, and W in accordance with existing symptoms (night cough, wheezing or dyspnea) at the time of bronchoscopy. The seven asymptomatic patients (S0) were defined as children without any symptoms for at least 1 mo before and after the fiberoptic

TABLE 1

INDICATIONS OF FIBEROPTIC BRONCHOSCOPY IN 72 CHILDREN

	A	CC	W	C	CF	Total
Radiological abnormalities						
Persistent parenchymal shadowing:	7	3	8	—	—	18
Asymmetrical lung fields:	1	—	4	—	—	5
Bronchial thickening*:	4	5	8	—	—	17
Suspected malformations						
Upper airways:	—	—	—	5	—	5
Intrathoracic airways:	—	—	1	2	—	3
Unexplained symptoms and signs						
Failure of treatment:	3	2	9†	—	—	12
Uncertain etiology††:	2	5	4	—	—	11
Suspected tuberculosis:	—	—	—	1	—	1
Psychogenic cough:	—	—	—	1	—	1
Stridor:	—	—	2	1	—	3
Hemoptysis:	1	1	—	—	—	2
Uncontrolled exacerbation:	—	—	—	—	10	10

Definition of abbreviations: A = asthma; C = control; CC = chronic cough; CF = cystic fibrosis; W = infantile wheeze.

* Associated with another indication in all of the children but one with a chronic cough, in whom bilateral bronchial thickening was marked.

† Associated in two cases with persistent radiological abnormalities.

†† Suspected extrinsic allergic alveolitis (n = 4), localized wheezing (n = 1), and unexplained symptoms (n = 6).

bronchoscopy. There were 13 children who suffered from at least weekly respiratory symptoms (S2), significantly affecting their quality of life (sleep, behavior, exercise tolerance, and school attendance). The remaining 32 symptomatic (S1) children had exercise-induced symptoms or mild to moderate exacerbations, with little effect on their quality of life. The children treated with inhaled steroids (5 in S0, 15 in S1, and 10 in S2) were not significantly different from each other ($\chi^2 = 4.47$, $p = 0.132$).

Bronchoscopy Procedure

All children were admitted to day ward and transnasal fiberoptic bronchoscopy (Olympus [Norwood, MA] BF 3C20 and Olympus BF P10 in the older children) was perfomed with monitoring by continuous oximetry and clinical evaluation. Premedication consisted of intramuscular atropine sulfate (0.01 to 0.02 mg/kg, and less than 1 mg), midazolam, and pethidine (14). Topical anesthesia of the upper and lower airways consisted of lidocaine, 2 and 0.5%, respectively. The bronchoscope was wedged in a segmental bronchus, the site depending on the chest radiographic abnormalities, most often in the right middle lobe. Aliquots of 10 ml of warmed sterile 0.9% saline solution were injected and aspirated, using suction. The total volume (3 ml/kg) varied from 20 to 60 ml according to the age and tolerance of the patient. The liquid recovered was pooled in a fresh sterile vial and divided into aliquots. To prevent possible contamination from the upper airways, no suction through the bronchoscope was used until the tip was wedged in the relevant segmental bronchus.

Microbiological Analysis

Quantitative cultures were carried out for growth of common aerobic agents such as *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Streptococcus* spp., and *Escherichia coli. Staphylococcus aureus and Pseudomonas* spp. were specifically sought in the CF group. A significant result was defined as growth $> 10^3$ CFU/ml.

Cell Counts

The unfiltered lavage fluid was centrifuged for 10 min at 1,800 rpm at 4° C. The pellet was resuspended in phosphate-buffered saline (PBS, 5 ml) containing 10% fetal calf serum. The total cell count was performed using a hemocytometer. Viability was assessed by the trypan blue exclusion test. Differential cell counts were carried out on Cytospin (Shandon, Pittsburgh, PA) slide preparations stained with May-Grünwald-Giemsa. At least 2 slides and 200 cells were counted for each subject.

Immunofluorescence

Samples were analyzed by two- or three-color flow cytometry, using the following antibodies: CD3-PECy5 (Caltag Laboratories, San Francisco, CA); and CD8-FITC (fluorescein isothiocyanate), CD19-FITC, CD4-PE (phycoerythrin), and CD2-PE (Coulter Immunology, Hialeah, FL). Briefly, cells were resuspended to 10^7/ml and 100 μl of BAL cells was incubated with antibody for 20 min at room temperature. The suspension

TABLE 2

CHARACTERISTICS OF THE 72 CHILDREN STUDIED

	A (*n* = 14)	CC (*n* = 12)	W (*n* = 26)	C (*n* = 10)	CF (*n* = 10)
Age (mo), median and range	85 (50–180)	51.7 (10–143)	24 (5–48)	97.8 (17–190)	78.5 (32–187)
Age at onset (mo), median and range	23 (1–150)	40.9 (1–140)	5.5 (1–30)	—	—
Sex ratio, M/F	11/3	10/2	13/13	5/5	4/6
Familial asthma history, %	50	33	34.5	30	0
Familial allergy history, %	36	50	38.5	10	10
Atopic dermatitis, %	36	25	11.5	0	0
Positive skin test, %	47	50	36*	—	—
High total IgE level, %	43	8	30	—	40
Proved allergy, %	50	25	—	—	40†
Symptom score, S0/S1/S2	4/8/2	0/9/3	3/15/8	—	—
Inhaled steroids	8	4	17	—	3 + 2††
Positive BAL culture/available	4/12	7/9	13/23	2/10	9/10
Haemophilus influenzae	1	3	7	2	3
Streptococcus pneumoniae	1	2	1	1	
Moraxella catarrhalis	2	1	3		
Streptococcus spp.	2	4	5	1	
Escherichia coli			1		1
Pseudomonas spp.					1
Staphylococcus aureus					2
Aspergillus fumigatus					3

Definition of abbreviations: A = asthma; C = control; CC = chronic cough; CF = cystic fibrosis; W = infantile wheeze.

* Data available in 14 infants.

† ABPA.

†† Two of the five children received oral steroids.

TABLE 3

BRONCHOALVEOLAR CELL PROFILES OF 72 CHILDREN WITH OBSTRUCTIVE DISEASES*

	Asthma ($n = 14$)	Chronic Cough ($n = 12$)	Infantile Wheeze ($n = 26$)	Control ($n = 10$)	Cystic Fibrosis ($n = 10$)
Viability, %	65 [54–72]	56 [38–63]	61 [50–76]	70 [51–79]	55 [51–72]
Total cells, 10^6/ml	0.521 [0.400–0.700]	0.630 [0.403–0.986]	0.705 [0.291–0.930]	0.490 [0.410–0.560]	1.015 [0.692–1.460]
Epithelial cells, 10^6/ml	0.080 [0.031–0.140]	0.058 [0.028–0.084]	0.044 [0.014–0.164]	0.25 [0.013–0.07]	0.078 [0.015–0.177]
Lymphocytes, 10^6/ml	0.299 [0.02–0.056]	0.025 [0.02–0.069]	0.025 [0.006–0.092]	0.031 [0.016–0.046]	0.068 [0.046–0.102]
Macrophages, 10^6/ml	0.342 [0.217–0.490]	0.434 [0.227–0.599]	0.252 [0.008–0.440]	0.348 [0.271–0.386]	0.625†§ [0.381–0.756]
Neutrophils, 10^6/ml	0.016 [0.005–0.719]	0.041 [0.008–0.057]	0.039 [0.009–0.278]	0.013 [0.008–0.018]	0.265†§ [0.168–0.448]
Eosinophils, 10^6/ml	0.013‡‖ [0–0.047]	0 [0–0.004]	0 [0–0.005]	0 [0–0.005]	0.023†§ [0–0.081]
CD4¶	16 (8–26)	18 (2–22.5)	22.3 (1–58)	—	—
CD8¶	42 (4–48)	42 (4–48)‖	40.5 (7.3–67)	—	—
CD4/CD8 ratio¶	0.266‡ (0.177–0.833)	0.428 (0.349–0.826)	0.455 (0.09–4.83)	—	—
CD19¶	2.2 (0.5–35)	6.5 (2.1–37)	4.5 (1–18)	—	—

* Data are expressed as median and [25th–75th quartile] or (range).
† $p < 0.05$ compared with children with asthma.
‡ $p < 0.05$ compared with infantile wheezers.
§ $p < 0.05$ compared with controls.
‖ $p < 0.05$ compared with children with chronic cough.
¶ Lymphocyte subsets were available in 9 children with asthma, 5 children with cough, and 16 infantile wheezers.

was then treated with red blood cell lysing solution (Q-Prep; Coulter Immunology). Using an EPICS-ELITE flow cytometer (Coulter Electronics, Hialeah, FL) equipped with a 15-mW argon laser, the cell surface markers were analyzed until 3,000 lymphocytes were counted.

Data Analysis

Cell counts were expressed as 10^6/ml or as a percentage of total cell counts (including the epithelial cells). Results are presented as means and standard deviation (± SD) as well as medians and interquartile ranges [25th to 75th].

Statistic Analysis

Correlations were made with Spearman's two-tailed rank correlation (Rho) in addition to Pearson's linear regression two-tailed analysis (r). Multiple linear regression analysis was used to study the relationship between total cell and differential cell counts. When applicable, differences between two groups were determined using the nonparametric Mann–Whitney U test. Differences between more than two groups were estimated by variance analysis (Kruskall–Wallis test) and by nonparametric tests. A p value of less than 0.05 was considered significant.

RESULTS

Patient characteristics are summarized in Table 2. The CC group had a notably high frequency of atopic family history and positive allergy skin prick tests. Preprocedure salbutamol nebulization was prescribed for 23 patients and four infants underwent the bronchoscopy while receiving oxygen supplementation (1 L/min) via a nasal cannula. Overall, the tolerance of the procedure was good. Four children had transitory hoarse cough and two others needed salbutamol nebulization after the procedure. No fever was reported. The mean (± SD) recovery of BAL fluid was 49 ± 19% and no difference was found between the groups. We found no difference in BAL fluid recovery with age and no relationship between cytology findings and age.

Differences between the Cell Profiles of Each Group

Viability and differential cytology results are summarized in Table 3. The greatest total cell number was in children with CF (Figure 1), in which the increase in the total cell number was proportional to a significant rise in both polymorphoneutrophils (PMNs) ($r = 0.801$, $p = 0.004$) and alveolar macrophages (AMs) ($r = 0.615$, $p = 0.05$). Eosinophils clearly characterized asthma (Figure 2) and were rare in infantile wheezers. They were found in 64% of children with asthma compared with 27% of infantile wheezers ($p = 0.04$) and 25% of children with chronic cough ($p = 0.06$). High percentages of eosinophils were also found in children with CF, which could be explained by the presence of allergic bronchopulmonary aspergillosis in four of them. Neutrophils were increased in children with CF but also in one third of the children with asthma and in one-half of the infantile wheeze (Figure 3). We, however, distinguished two distinct, equivalent subpopulations of infantile wheezers composed of 13 children with a proportion of neutrophils < 10% and 13 subjects with a high proportion, ranging from 16 to 80%.

The macrophage ratio in controls was similar to that in the chronic cough group but higher than in the asthma and infantile wheeze groups (Figure 4). A high epithelial cell ratio, suggesting epithelial shedding, was found in half of the infantile wheezers and in half of the children with asthma, who differed significantly from children with CF. No variation in the lymphocyte and basophil cell counts was found between any of the groups. Medians [25th–75th quartiles] of lymphocyte percentages were as follows: 6.5 [4–10] in asthma, 6 [4–8.5] in chronic cough, 8 [4–9] in infantile wheeze, 7 [5–8] in controls and 7 [4–7.5] in CF. Basophils were detected (1% of total cells) in two children with asthma, two with chronic cough, and two infantile wheezers. No key cell type was observed in the chronic cough group, for which the cell profile appeared similar to that of the control group and, therefore, distinct from both asthma and infantile wheeze groups.

The influence of steroids was studied by comparing the total and differential cell counts between the treated and untreated children in the groups, when such a statistical analysis was possible (asthma, infantile wheeze, pooled A-CC-W group, and cystic fibrosis). The median [25th–75th quartile] lympho-

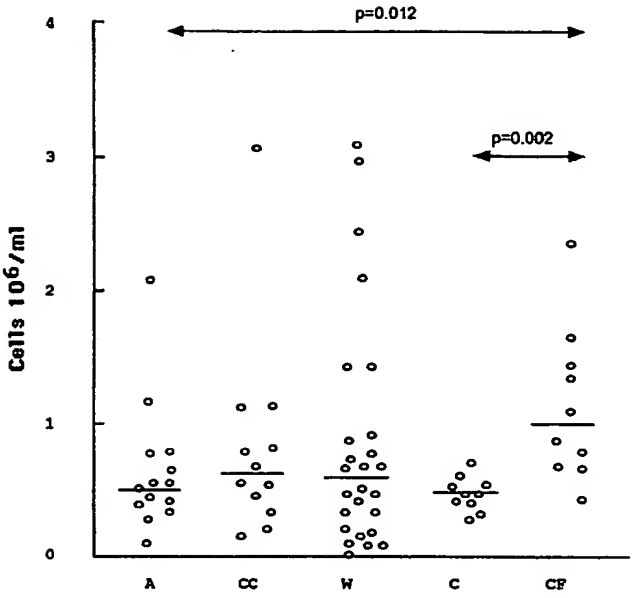

Figure 1. Total cell numbers in children with asthma (*A*, n = 14), chronic cough (*CC*, n = 12), infantile wheeze (*W*, n = 26), and cystic fibrosis (*CF*, n = 10) and in control children (*C*, n = 10). *Horizontal bars* indicate the median for each group of patients.

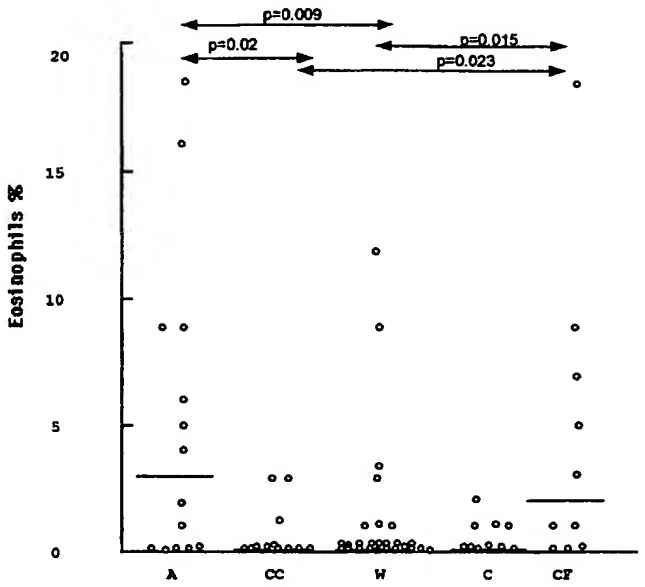

Figure 2. Eosinophils as a percentage of total cells in children with asthma (*A*, n = 14), chronic cough (*CC*, n = 12), infantile wheeze (*W*, n = 26), and cystic fibrosis (*CF*, n = 10) and in control children (*C*, n = 10). *Horizontal bars* indicate the median for each group of patients.

cyte ratio in CF was higher in children receiving steroids than in those not receiving steroids: 7 [7–9] and 4 [3–5]% (p = 0.018), respectively. A difference in the other groups was found neither in total cell counts nor in differential cell counts. Considering the key cell types that we individualized, medians [25th–75th quartiles], in treated and untreated children, of the eosinophil ratio in asthma were 2 [0–5] and 4 [0.5–12.5]% (p = 0.321) and of the neutrophil ratio in infantile wheeze were 12.5 [4 to 52.5] and 8.5 [2–50]% (p = 0.781), respectively.

Microbiological Culture

The proportions of cultures positive for the most commonly encountered bacteria are shown in Table 2. The number of positive BAL cultures was significantly greater in the chronic cough and infantile wheeze groups compared with the control group (p = 0.02). Two patients in the control group had unexpected positive BAL cultures but did not have any macroscopic evidence of lower airway inflammation or elevated neutrophil numbers. We believe this represents the false-positive bacterial culture rate of BAL using our technique in small children. Despite our best efforts, occasional contamination from the upper airway is inevitable. The neutrophil ratio was significantly higher when combined BAL cultures (i.e., representing children with asthma, cough, and infantile wheeze) were positive than when BAL cultures were negative: 26 ± 7 and 12 ± 4% (p = 0.014), respectively (Figure 5). Among wheezing infants alone, a low neutrophil ratio (< 10%) was more frequent in the negative BAL culture group than in the positive BAL culture group: 75 versus 33% (p = 0.057), respectively.

Influence of Persistent Radiographic Shadows on Neutrophil Counts and BAL Cultures

Those children with asthma, cough, or infantile wheeze, and having persistent radiological abnormalities, are listed in Table 1. For the analysis, chest X-ray features were classified in three groups: normal (n = 12), persistent parenchymal shadowing (n = 18), and others (n = 22). No significant difference between these three groups was established either in the percentage of neutrophils (12.4 ± 16.3, 20.3 ± 23.8, and 19.6 ± 27.9%, respectively), or in the number of children with a positive BAL culture (44, 64, and 59%, respectively). Further analysis of asthma, chronic cough, and infantile wheeze subpopulations showed no relationship between the neutrophil ratio and the radiological features. A high ratio of neutrophils was found in 41% of the infantile wheezers and 37.5% of the children with asthma with persistent shadowing.

Differential Cell Counts and Symptomatic Status in Asthma, Chronic Cough, and Infantile Wheeze

The total cell number and the ratio of neutrophils were related to the symptom score (Figure 6). The children with more severe disease had a higher total cell number and the presence of symptoms was associated with an increase in neutrophils. Covariate analysis showed that this relationship between the PMN ratio and symptoms was independent of the radiological features (p = 0.540).

Lymphocyte Subpopulations

Lymphocyte subpopulations were analyzed in 9 children with asthma, 5 children with chronic cough, and 15 wheezing infants (Table 3). The median (range) values of the CD2 and CD3 percentages were as follows: 84 (57–95) and 78.5 (63–81.4) in asthma, 65.6 (48–83.5) and 56 (49–63) in chronic cough, 76 (14.4–94) and 78 (10.5–90) in infantile wheeze. The ratio of $CD8^+$ cells was higher in children with asthma (p = 0.027) than in wheezing infants. The cell surface markers were not related to age, symptom score, or treatment with steroids.

DISCUSSION

In this study, we present cell profiles of BAL fluid from infants and children with asthma, infantile wheeze, or chronic

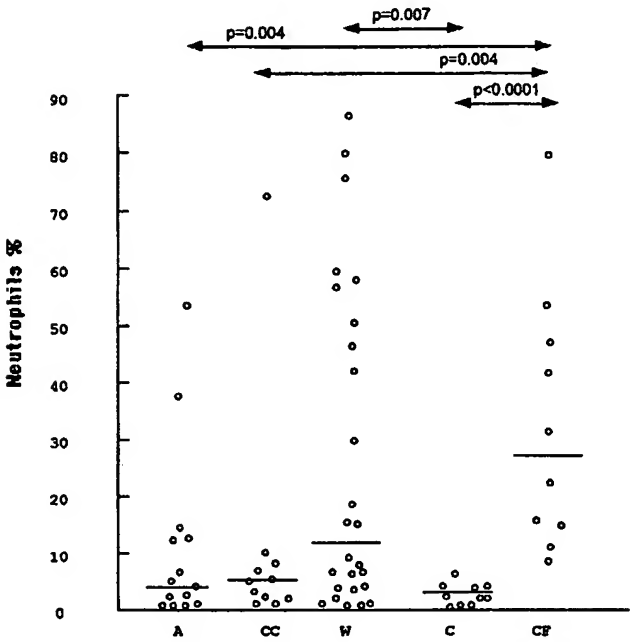

Figure 3. Neutrophils as a percentage of total cells in children with asthma (*A*, n = 14), chronic cough (*CC*, n = 12), infantile wheeze (*W*, n = 26), and cystic fibrosis (*CF*, n = 10) and in control children (*C*, n = 10). *Horizontal bars* indicate the median for each group of patients.

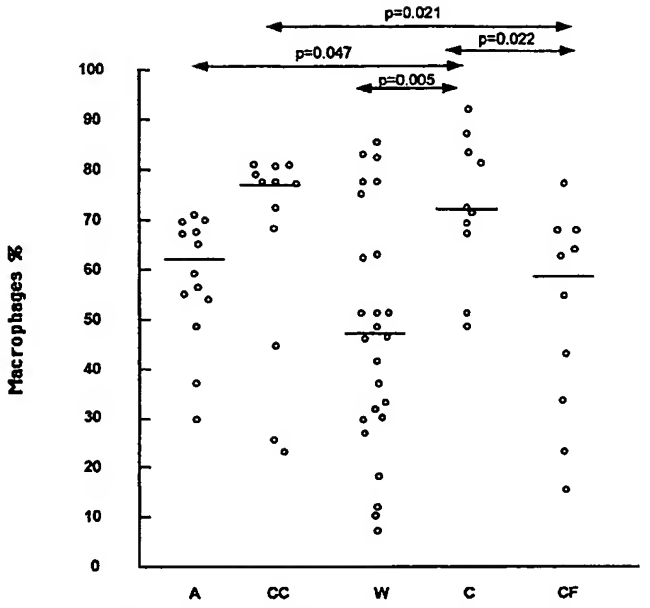

Figure 4. Macrophages as a percentage of total cells in children with asthma (*A*, n = 14), chronic cough (*CC*, n = 12), infantile wheeze (*W*, n = 26), and cystic fibrosis (*CF*, n = 10) and in control children (*C*, n = 10). *Horizontal bars* indicate the median for each group of patients.

cough. Children with cystic fibrosis and another group of children with no obvious lower airway disease, in whom a different pathophysiology was expected, were also studied. We adjusted the bronchoalveolar lavage volume to body weight, which has been shown to yield a constant fraction of epithelial lining fluid in healthy children (6, 15), and the results were expressed per milliliter of the recovered fluid (3). We chose not to use dilution markers, as they depend on the procedure and have been considered unreliable in stable asthmatics or when the epithelial permeability was likely to be modified by inflammation (16). The recovery of BAL fluid in our study was lower than those reported in healthy children (4–5) but similar to some studies (10–12) and rather better than the 30% yield from nonbronchoscopy-obtained BAL in wheezy children (8).

The processing of bronchoalveolar lavage should also be considered when cellular components are analyzed or compared with data from other research groups. The site of the lavage does not modify the cytology findings (7). On the other hand, total cell and neutrophil counts vary depending on whether the first aliquot is included or discarded (5, 17). Epithelial cell counts are known to be underestimated when BAL fluid is subjected to gauze filtration (18). As this study concerned children with chronic bronchial diseases, in which epithelial cells might be involved, we opted to use no lavage filtration and pooled the aliquots (6, 16). Therefore, as found by Heaney and co-workers (6), we reported high percentages of epithelial cells and a relative poor overall cell viability in comparison with those established in other studies. The differential counts have been suggested to be related to age (4, 7), but in accord with other studies (5, 6) we found no relationship between age and differential counts, which depended on the underlying disease.

All groups that we studied were characterized by a higher total cell number than in healthy children (Table 4) and by a constant lymphocyte ratio, which was comparable to those reported in healthy children (4) and infants with miscellaneous airway diseases (7). Although our control group comprised children in whom fiberoptic bronchoscopy was justified by respiratory problems, the median differential cell counts were close to those found in pooled BAL from healthy children (6).

We demonstrated that asthma in childhood is characterized

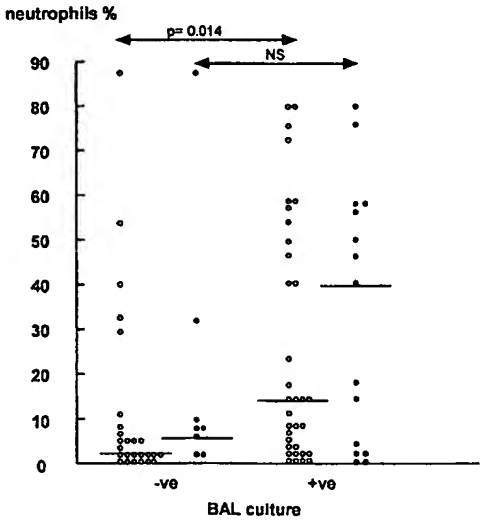

Figure 5. Neutrophils as a percentage of total cells in relation to microbial colonization of BAL in control children and children with asthma, chronic cough, and infantile wheeze (*open circles*) and in children with infantile wheeze (*solid circles*). *Horizontal bars* indicate the median for each group of patients.

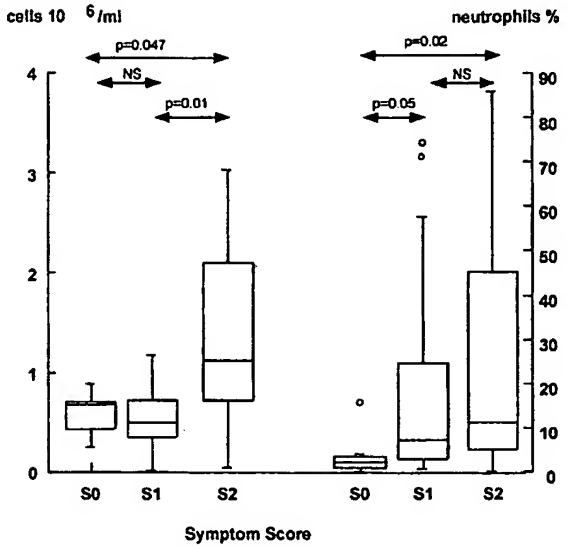

Figure 6. Neutrophil ratio and total cell numbers in relation to the severity of symptoms in 52 children with asthma, chronic cough, or infantile wheeze. *S0* (n = 7), asymptomatic in the month before and after bronchoscopy lavage; *S1* (n = 32), mild or moderate symptoms; *S2* (n = 13), severe symptoms. Results are expressed as box and whisker plots and outlier values are displayed.

by the presence of eosinophils and a high proportion of epithelial cells. Recognized as key cells of inflammation in adult asthma (3), eosinophils were previously found in BAL fluid from children with asthma (Table 4), but two of the three studies were conducted after a provocation challenge (10, 12). In our study, the proportion of eosinophils was independent of the severity of the disease and of treatment with steroids. Epithelial shedding has been considered specific to asthma (19), and was suggested by the high epithelial ratio encountered in our children with asthma. This ratio was higher than in cystic fibrosis, in which neutrophil counts were the highest. This indicates that epithelial shedding is not necessarily characteristic of neutrophil-mediated inflammation. As expected, the cystic fibrosis group was distinguished from the other groups by high cellularity and neutrophil ratio (20) and, surprisingly, by an elevated ratio of eosinophils, which could be explained in this study by the presence of allergic bronchopulmonary aspergillosis (ABTA) (11). However, raised serum eosinophil cationic protein levels have been described in patients with CF with exacerbation of infection in the absence of ABPA (21).

The significance of chronic cough, which occurs frequently with asthma, depends on whether it is associated with wheezing (13). We distinguished children with chronic cough and no wheezing, in whom the differential cell counts were comparable to those observed in healthy and control children, implying a cellular pathophysiology different from that of asthma. Infantile wheezers were a heterogeneous group and some would be expected to become asthmatic (22). As previously described (8, 9), we occasionally found raised eosinophil counts but a high proportion of epithelial cells. This suggests that epithelial damage might occur independent of eosinophilic inflammation. Inhaled steroids had no influence on the cell profile findings in our study, whereas a decrease in number of the recruited lymphocytes, eosinophils, and mast cells would be expected (23). However, numbers were small and an antiinflammatory effect cannot be excluded because we did not perform BAL before and after commencing inhaled steroids.

We compared the lymphocyte subset counts in asthma, chronic cough, and infantile wheeze groups with values obtained in healthy children (5, 24). The number of cells per milliliter was larger in our study, with three times more lymphocytes in all of the groups. Nonetheless, the rise did not occur in the same proportion in $CD4^+$ and $CD8^+$ subsets. Consequently, in comparison with those determined in healthy children, the CD4/CD8 ratio was lower. Similar shifts of $CD4^+$ and $CD8^+$ lymphocytes have been described in asthmatic adults (3). An effect of recurrent viral infections has been suggested, but this remains unclear (25, 26); we showed a higher proportion of $CD8^+$ cells in wheezing infants, whereas syncytial respiratory virus is known to increase the CD4/CD8 ratio in BAL fluid (27).

TABLE 4

BRONCHOALVEOLAR CELL PROFILES OF CHILDREN IN OTHER STUDIES*

Population	Ref.	n	First Aliquot	Percentage of Recovery	Viability (%)	Total Cells	AM	PMN	Ly	Eo	Ep. Cells
Healthy children†	4	48	D	58 ± 15	78	73	64	1	10	—	—
							(84)	(0.9)	(12.5)	(0.2)	—
	5	18	D	65	94	155	120	2.3	9	0.1	0.3
							(91)	(1.7)	(7.5)	(0.2)	(0.3)
	6	55	P	35 ± 1.4	—	95	(70)	(3.2)	(3)	(0.09)	(13.4)
Control infants†	7	16	D	43 ± 3	—	510	(87)	(3.5)	(7)	(0)	—
	9	7	D	—	74	300	(91)	(0.8)	(4)	(0)	(2.1)
Control children‡	11	24	D	30–60	90	5.2§	(89)	(4.2)	(3.9)	(0.7)	(2)
Children with asthma‡	11	15	D	30–60	89	2.3§	(31)	—	(5.6)	(4.8)	(3.3)
	10	22	P	38	—	144	113	18	5	7.7	—
	12	17	P	41	—	176	148	5.9	5.1	16.1	—
Children with atopic asthma†	8	52	P	30	—	83	(71.3)	(3.5)	(1.4)	(1.1)	(14.1)
Infantile wheezers†	9	13	D	—	72	380	(85)	(3.2)	(4.5)	(0)	(7.2)
	8	14	P	30	—	136	(53.6)	(9)	(2.3)	(0.2)	(24/1)

Definition of abbreviations: AM = macrophages; D = discarded; Eo = eosinophils; Ep. = epithelial; Ly = lymphocytes; P = pooled; PMN = neutrophils.

* The total number of cells and differential cell counts are expressed as 10^3/ml (unless otherwise noted) or as a percentage of total cells (indicated in parentheses).

† Median values are displayed as indicated.

‡ Mean values are displayed as indicated.

§ Expressed as 10^6/ml.

One of the main findings in our study was the increase in neutrophils in one-half of the wheezing infants and in one-third of the children with asthma. This is different from the findings of one study of children with allergy or virus-associated wheeze, but techniques and recruitment of subjects did differ (8). Although neutrophil counts in adults with airway diseases were often similar to those of controls (23), some studies have suggested a role for neutrophils in bronchial inflammation (20). As in our study, their recruitment was related to the activity and severity of asthma (28, 29).

We provided evidence of the contribution of neutrophils to the pathophysiology of airway disease by showing a raised percentage in the symptomatic children, even when they were treated with inhaled steroids. Such a predominance of neutrophils has been attributed to lung infection in children with a middle lobe syndrome (30). To a certain extent, our study supports the concept that bacterial infections induce recruitment of neutrophils to the airways but this occurs irrespective of the radiological features. Thus occult bacterial colonization or infection may be causing the persistence of neutrophils in the airway lumen. However, viruses are considered to be much more relevant to the pathogenesis of asthma and infantile wheeze (31). A predominance of neutrophils has been shown in BAL fluid from infants with syncytial respiratory virus bronchiolitis (27). Furthermore, bacterial adherence is enhanced by viral infections, which both activate and/or damage the epithelial cells (32).

It is possible that allergen-induced late-phase responses in the airways might have contributed to neutrophil recruitment (3). Neutrophils may be attracted to the sites of inflammation by a range of chemotactic proteins released from a range of cells (20). Both high and low molecular weight neutrophil chemotactic mediators have been demonstrated to be released during allergen-induced responses and also during acute asthma (28, 33). Furthermore, neutrophils do have the potential to release a wide range of factors that could contribute to the prolonged inflammation and hyperreactivity associated with asthma (3, 20, 34).

Alveolar macrophages are important in the regulation of neutrophil recruitment, releasing both neutrophil chemoattractants (i.e., interleukin 8) and inhibiting factors (20). Thus, the macrophages were shown to be activated in cystic fibrosis (35) and in infantile wheeze (9). However, the analysis of cellular components of BAL fluid suggested a different mechanism of recruitment of cells in cystic fibrosis compared with asthma and infantile wheeze. In cystic fibrosis (35), recruited macrophages accompanied the rise in neutrophils, both being increased early in the first months of life, as soon as bacterial infection occurred (36). Conversely, the elevated neutrophil number in wheezing infants and children with asthma was related to a diminished macrophage number, as previously described in chronic lung disease of prematurity (37), where infection (38) and neutrophil recruitment were also relevant (39).

In conclusion, we have shown that obstructive diseases in childhood had variations in differential cell profiles implying different inflammatory responses. The variation within the chronic cough and infantile wheeze groups almost certainly reflects the different outcomes in these children, some of whom will become asthmatic. However, the children with chronic cough were in general indistinguishable from controls in relation to cell profiles. This suggests that the majority are not destined to be asthmatic and should not be treated by anti-asthma prophylaxis. We have demonstrated that neutrophils played a more prominent role in childhood wheezing illnesses than is suggested by adult studies, and this was associated with more frequent bacterial airway colonization. Whether processes other than bacterial infection, such as virus infection or allergic responses, contribute to neutrophil recruitment remains to be established. However, it does raise the question as to whether antibiotics might contribute to the management of early wheeze. We do not know whether the persistence of neutrophils in the intralumenal airways contributes only to current symptoms or to the subsequent development of asthma. Study of BAL fluid in children with evolving airway diseases is improving our understanding of immunopathology, but follow-up of the children investigated will be essential to elaborate the significance of our findings.

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